Pakistan J. Zool., vol. XX(0), pp 000-000, 2024 **DOI**: https://dx.doi.org/10.17582/journal.pjz/20231225161301

Effects of Dietary Omega-3 Fatty Acids on Reproductive Performance and Biochemical Parameters of Lactating Cows in Arid Subtropics

Abd El-Nasser Ahmed Mohammed[1]*, Mohammed Al-Saiady[2], Ahmed El-Waziry[3] and Tarek Al-Shaheen[1]

[1]Department of Animal and Fish Production, College of Agricultural and Food Sciences, King Faisal University, P.O. Box 420, Al-Hassa 31982, Kingdom of Saudi Arabia
[2]ARASCO Research and Development Department, P.O. Box 53845, Riyadh 11593, Kingdom of Saudi Arabia
[3]Department of Animal and Fish Production, Faculty of Agriculture, El-Shatby, Alexandria University, P.O. Box 21454, Egypt.

Article Information
Received 25 December 2023
Revised 02 February 2023
Accepted 13 February 2024
Available online 03 May 2024
(early access)

Authors' Contribution
MA designed the study. TA carried out the study and collected the data. AE statistically analyzed the results. AA wrote the manuscript. All authors interpreted the data, revised the manuscript, and approved the final version.

Key words
Fatty acids, Extruded flaxseed, Salmate, Estrous, Conception, Metabolites

ABSTRACT

Dietary omega-3 fatty acids are a type of polyunsaturated fat known to improve production and body health in mammalian species. The present on-farm experiment was designed to evaluate the effects of omega-3 fatty acids sources, extruded flaxseed and salmate, on reproductive performance and biochemical parameters in lactating dairy cattle. Two hundred and sixty-eight lactating cows were blocked by stage of lactation and assigned to three groups, a control group and two treated groups fed diets containing salmate (25 g/head/day) and extruded flaxseed (7.0%). The formulated control, salmate and extruded flaxseed diets were isoenergetic and isonitrogenous. The diets were given to animals from three weeks pre-partum to 160 days postpartum. Reproductive performance and biochemical parameters were investigated in both control and treated groups. The results indicated earlier restoration of normal oestrous postpartum in flaxseed group (17/90, 18.89%) followed by salmate (13/87, 14.94%) and control (3/91, 3.30%) groups. Upon estrous synchronization of remaining not estrous cows, the pregnancy rates were the highest in flaxseed (72/90, 80.0%) followed by salmate (67/87, 77.01%) and control (49/91, 53.85%) groups. The values of total protein, globulin, blood urea nitrogen, beta-hydroxybutyrate, cholesterol, thyroxine, IGF and insulin were higher in extruded flaxseed group compared to salamte and control ones versus NEFA, glucose, triglycerides and cortisol values. In conclusion, supplementation of flaxseed and salmate provided beneficial roles to lactating dairy cows in relation to reproductive performances and biochemical parameters in arid subtropical conditions.

INTRODUCTION

Dietary supplements to ruminant species affect both reproductive and blood metabolites (Al-Suwaiegh *et al*., 2022). The global requirements of milk and meat are predicted to increase to 35.0% by 2030 due to increase in population. The national systems in KSA support the commercial production of meat and milk production to overcome the increase of demands (Mohammed and Al-Suwaiegh, 2023). Therefore, several strategies have been explored a worldwide including dietary supplements that can improve body health and reproductive performance resulting in significant improvement of meat and milk production.

Lactating dairy cows undergo major endocrine and metabolic disorders during the transition period, which may have a negative impact on productive and reproductive performance (Gross and Bruckmaier, 2019; Pinedo and Melendez, 2022). Energy deficiency usually increases lipid mobilization from body reserves and intensive ketogenic and lipogenesis in the liver occur in cows after parturition and during the first couple of weeks of lactation due to increased activity of the mammary glands (Djokovic *et al*., 2013; Pinedo and Melendez, 2022). Therefore, several strategies were applied to ruminant animals during transitional period to alleviate and overcome the negative effects.

* Corresponding author: aamohammed@kfu.edu.sa
0030-9923/2024/0001-0001 $ 9.00/0

Feeding high protein diets increase blood urea nitrogen and consequently can reduce fertility of lactating dairy cows. On the other hand, low protein diets may have a negative impact on fertility and milk production (Underwood *et al*., 2022) through delayed estrus and lower conception rates. Feeding flaxseed and long-chain fatty acids are reported to have major impact on ruminant animal production and reproduction as a strategy to maintain both productivity and fertility (Karageorgou *et al*., 2023; Elbarbary *et al*., 2023; Dere Yelken *et al*., 2024). Quality and viability of embryos, as well as the general reproductive performance are increased through their addition into diets (Petit *et al*., 2008; Leroy *et al*., 2013). At early lactation, major changes occur in the animal's body metabolites (Al-Mufarji *et al*., 2022, 2023). Additionally, major changes occur in the hormonal regulation of metabolic functions (Veshkini *et al*., 2023). Negative energy balance (NEB) during early lactation usually leads to deterioration of organic matter metabolism and animals may depend on body reserves to meet energy demand (Ali *et al*., 2021). The levels of triiodothyronine and thyroxine are considered indicators of adaptation to NEB until energy balance is maintained (Senosy *et al*., 2017). Beta-hydroxybutyrate is the most important and abundant ketone body and considered the main blood indicator of ketosis and lipid mobilization in ruminants. Non-esterified fatty acid (NEFA) is also an indicator of ketosis (Senosy *et al*., 2017, 2018). Most experiments on flaxseed were short-term trials during lactation stage; therefore, there is a need to provide more information on feeding extruded flaxseed for longer periods. The present study starts three weeks before parturition and extend to week 23 of lactation using two hundred and sixty-eight lactating Holstein cows. Reproductive performance and metabolic profiles during this long period were evaluated. The hypothesis is to shorten open days, improve reproductive performance and blood metabolites and hormones of high yielding dairy cows in arid subtropics.

MATERIALS AND METHODS

Diets and animals management

The present trial was conducted in a dairy farm located in Riyadh of KSA from June to December 2023. Two hundred and sixty-eight lactating cows were used for the trial. The cows had 582.0±21.0 kg of body weight and 3.35±0.10 of body condition score. They were divided through complete randomized design into three groups based on parity and milk yield. The cows were randomly distributed for investigating the effects of extruded flaxseed (7.0%) and salmate (25g/head/day) compared to control diet. The average relative humidity ranged from 8.0 to 94.0 and ambient temperature ranged from 31.0 to 48.0. It is important to indicate that all cows were kept under evaporative cooling system, which works during stressful condition. Animals were placed in an open lot. The period of experiment lasted six months. The control, salmate and extruded flaxseed diets were formulated to provide the nutrient requirements of lactating dairy cows according to body weight, body gain (0.2 kg day^{-1}), milk production and milk fat (3.5%) as recommended by NRC (2001). The cows were given fresh water and mineral mixture *ad libitum*. The diets of salmate and extruded flaxseed were weekly prepared to avoid lipid peroxidation.

Extruded flaxseed and salmate supplements

Ingredients of extruded flaxseed include 85 g/kg flaxseed and 15 g/kg wheat bran (ARASCO, KSA). The salmate is dried fish oil contains 31% omega- 3 and 5% Omega-6 fatty acids. The extruded flaxseed contains 28.0% fiber, 44.0 fat, 21.0% protein, 6.0% carbohydrates and 4.0% ash. In addition, the extruded flaxseed fat contains 47.0% omega-3 fatty acid and 15.0% omega 6 fatty acid.

Reproductive parameters

Animals of each group were followed for estrous detection each three weeks post-calving for three consecutive days. In addition, estrus cycles were synchronized using protocol of GnRH + PGF2α + GnRH (Kesler, 2005). Reproductive parameters were evaluated through heat detection, open days, number of services per conception and conception rates.

Blood samples and analysis

Blood samples were collected weekly from 10 cows of each group through tail vein puncture into two vacutainer tubes; one tube containing anticoagulant and the other one without anticoagulant for separation serum and plasma, respectively. Serum and plasma were stored at -20°C until further analysis. The serum samples were analyzed for total protein (TP), albumen, globulin, and blood urea nitrogen (BUN) using commercial kits (Randox Lab., United Kingdom). Plasma concentrations of glucose, β-hydroxybutyrate acid (β-HBA), total cholesterol, triglyceride (TG), cholesterol, high-density lipoprotein (HDL) and non-esterified fatty acids (NEFA) were determined spectrophotometrically using commercial kits (Randox Lab., United Kingdom). Acetate was determined by colorimetric method using commercial kits (Biovision incorporated, USA). Glycerol was determined by colorimetric method using commercial kits (Cayman Chemical Company, Ann Arbor, MI, USA). Insulin was determined by indirect immunoassay technique using commercial kit (Alpco Company, NH, USA). Plasma

triiodothyronine (T3), thyroxine (T4) and cortisol levels were measured using commercial ELISA kits (Human, Germany), and micro-titrimetric plates. The assay procedures were done according to the manufacturer's instructions, and the absorbance values were done by automatic photometer.

Statistical analysis

Data of blood metabolites (TP, albumin, globulin, BUN, glucose, cholesterol, TG, β-HBA), hormones (Insulin, cortisol, T3 and T4) and reproductive performances (conception rate) were statistically analyzed by general linear model procedure of SAS (2008) using the following model; $Y_{ijk} = \mu + A_i + Bj + ABij + E_{ijk}$; where Y_{ij}, the observation; A, the effect of factorA; B, the effect of factor B; ABij, the interaction between factor A and B; E_{ijk}, standard error. Duncan's Multiple Range Test (1955) was used to compare the means of the control and treated groups.

RESULTS

Tables I and II shows that the effect of extruded flaxseed (7.0 %) and salmate (25 g/h/d dry protected fish oil) diets on reproductive performance, blood metabolites and hormones values of lactating dairy cows. The results support improvement of reproductive performance and body health conditions.

Table I. Effect of salmate and extruded flaxseed diets on reproductive performance parameters of lactating Holstein cows.

Periods	Control	Flaxseed	Salmate
No. of cows	91	90	87
Normal estrous, no. (%)	3 (3.3%)	17 (18.89%)	13 (14.94%)
Synchronized estrous, no. (%)	88 (96.70%)	73 (81.11%)	74 (85.06%)
Number of service/conception	2.52±0.17	2.63±0.14	2.58±0.15
Open days	106.23 ± 3.91	102.22 ± 4.95	101.15 ± 4.80
Pregnancy, % (n)	53.85% (49/91)	80.0% (72/90)	77.01% (67/87)

Control group fed basal diet. Extruded flaxseed group fed isoenergetic and isonitrogenous diet containing 7% extruded flaxseed. Salmate group fed isoenergetic and isonitrogenous diet containing 25 g/head/day of dried fish oil.

Table II. Effects of dietary extruded and salmate on blood metabolites of lactating dairy cattle.

Items	Control	Treatment		SEM	P-value		
		Flaxseed	Salmate		Treat	Period	Treat x period
Total protein (g/dL)	6.700^{ab}	6.720^{a}	6.600^{b}	0.052	0.0880	<0.0001	<0.0002
Albumin (g/dL)	3.870	3.830	3.850	0.016	0.3604	<0.0001	0.3300
Globulin (g/dL)	2.820^{ab}	2.890^{a}	2.750^{b}	0.053	0.1137	<0.0001	<0.0008
Urea nitrogen (mg/dL)	15.20^{b}	16.02^{a}	15.75^{a}	0.252	0.0016	<0.0001	<0.0001
NEFA (mmol/ml)	0.400^{a}	0.240^{b}	0.380^{a}	0.017	<0.0001	<0.0001	<0.0001
βHBA (mmol/ml)	0.460^{b}	0.560^{a}	0.480^{b}	0.010	<0.0001	<0.0001	<0.0001
Glucose (mg/dL)	63.72^{a}	58.54^{b}	59.5^{b}	0.437	<0.0001	<0.0001	<0.0001
Triglycerides (mg/dL)	34.29^{a}	18.60^{b}	20.02^{b}	0.546	<0.0001	<0.0001	<0.0001
Glycerol (mg/L)	8.830^{a}	5.930^{b}	4.360^{c}	0.014	<0.0001	<0.0001	<0.0001
Cholesterol (mg/dL)	152.75^{c}	200.18^{a}	170.66^{b}	3.488	<0.0001	<0.0001	<0.0001
HDL (mg/dL)	104.38^{b}	116.30^{a}	120.30^{a}	3.20	<0.0001	<0.0001	<0.0001
LDL (mg/dL)	20.16^{c}	57.84^{a}	29.28^{b}	0.251	<0.0001	<0.0001	<0.0001
Acetate (nmol/ml)	0.470^{b}	0.660^{a}	0.64^{a}	0.010	<0.0001	<0.0001	<0.0001
T4 (ng/dL)	2.200^{b}	2.360^{a}	1.980^{c}	0.040	<0.0001	0.0136	<0.0001
T3 (ng/ml)	0.950^{a}	0.950^{a}	0.800^{b}	0.008	<0.0001	<0.0001	<0.0001
Cortisol (ng/ml)	20.20^{a}	14.07^{b}	22.40^{a}	0.541	<0.0001	<0.0001	<0.0001
IGF (ng/ml)	3.820^{c}	5.830^{a}	4.400^{b}	0.089	<0.0001	0.0145	0.0220
Insulin (ng/ml)	0.410^{c}	0.650^{a}	0.540^{b}	0.014	<0.0001	<0.0001	<0.0001

$^{a, b, \text{and } c}$; values in the same row with different superscripts differ significantly ($P < 0.05$). Control group fed basal diet. Extruded flaxseed group fed isoenergetic and isonitrogenous diet containing 7% extruded flaxseed. Salmate group fed isoenergetic and isonitrogenous diet containing 25 g/head/day of dried fish oil. NEFA, Non-esterified fatty acids; βHBA, β-hydroxybutyrate acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein; T3, triiodothyronine; T4, thyroxine; IGF, Insulin-like growth factor-1.

Reproductive performance

Table I shows that extruded flaxseed and salmate diets restored earlier ovarian activity postpartum where 18.89% (17/90) and 14.94% (13/87) of cows were in normal heat compared to 3.3% (3/91) of control diet. Furthermore, the conception rate (%) of normal and synchronized estrous cows were the highest in extruded flaxseed group (80.0%; 72/90) followed by salmate (77.01; 67/87) and control groups (53.85%; 49/91). In addition, the number of services per conception and open days were approximately similar among groups.

Blood biochemistry profiles

The blood biochemistry indices showed that TP (g/dl) were the highest (6.72; P=0.088) in extruded flaxseed group compared to salmate (6.66) and control (6.70) ones. Simultaneously, globulin 2.89 vs. 2.75 and 2.82 g/dl), BUN (16.02 vs. 15.75 and 15.20 mg/dl), β-HBA (0.56 vs. 0.48 and 0.46 mmol/ml), cholesterol (200.18 vs. 170.66 and 152.75 mg/dl), acetate (0.66 vs. 0.64 and 0.47 nmol/ml), T4 (2.36 vs. 1.98 and 2.20 ng/dl), IGF (5.83 vs. 4.40 and 3.82 ng/ml), and insulin (0.65 vs. 0.54 and 0.410 ng/ml) values were followed the same trends. On the other hand, NEFA (mmol/l) were the lowest (0.24; P=<0.0001) in extruded flaxseed group compared to salmate (0.38) and control (0.40) ones as glucose (58.54 vs. 59.5 and 63.72 mg/dl), cortisol (14.07 vs. 22.40 and 20.20 ng/ml) values.

DISCUSSION

Omega-3 and omega-6 families should be supplied in the diets because mammalian species cannot synthesize those fatty acids in their bodies. In addition, the supplied feed additives must be safe for body health and wellbeing of pregnant and lactating animals to support their ovarian follicles' development, milk production and composition (Al-Mufarji *et al*., 2023; Al-Masruri *et al*., 2022). The relationships among nutrition, reproductive performances and metabolic body health conditions have been explored in several studies (Moallem, 2018; Ali *et al*., 2021; Al-Mufarji *et al*., 2022, 2023; Mohammed and Al-Suwaiegh, 2023; Di Meo *et al*., 2023). The current trial is long experiment conducted in commercial lactation farm to restore the negative effects of peripartum and postpartum periods in lactating Holstein cows. We present here part of results concerning reproductive traits and the related plasma metabolites and hormones (Tables I, II). The beneficial effects of omega-3 fatty acid on productive and reproductive performances and body health status were confirmed earlier in several short studies, which were differed in diet formulation and experimental conditions (Kra *et al*., 2021; Di Meo *et al*., 2023). The improvement in reproductive performances and body health conditions in the current was highest in extruded flaxseed (7.0%) group if compared to salmate (25g/h/d) and control groups.

The results of supplementing cows with extruded flaxseed, the most widely available botanical source of n-3 FA, and salmate (dried fish oil) showed notable improvement in reproductive performance concerning earlier restoration of ovarian activities and higher conception rates. The FAs of extruded flaxseed and salmate might affect the ovarian structures development and quality, fertilization rate and further embryonic development (Petit, 2002; Ambrose *et al*., 2006; Moallem, 2018). Santos *et al*. (2008) and Cerri *et al*. (2009) concluded that increased intake of polyunsaturated fatty acids (PUFs) might affect the fatty acid composition of reproductive tissues and consequently improve fertilization rate and embryonic development.

It has been found that cows supplemented with encapsulated flaxseed at 3.8% of DM had higher numbers of 2- to 5-mm follicles on d 5 and 9 of the estrous cycle (Zachut *et al*., 2010). In addition, cows supplemented with extruded flaxseed from 3 weeks pre-partum to 100 days postpartum had longer durations of estrous behavior and longer estrogen surge (Zachut *et al*., 2011). Robinson *et al*. (2002) found higher plasma E2 concentration in cows fed α-linolenic acid than in those fed linoleic acid. Holstein cows supplemented with rolled flaxseed and rolled sunflower for 32 d after insemination had larger ovulatory follicles and reduced pregnancy losses (9.8% vs. 27.3%) (Ambrose *et al*., 2006).

Beyond ovarian follicle development, Petit and Benchaar (2007) found a higher conception rate at first artificial insemination in cows supplemented with whole flaxseed (54.30%) than those fed with micronized soybeans (40.0%), which might be attributed to larger well-developed corpora lutea as found in cows fed diets supplemented with fish oil or line seed (Petit, 2002). In a large-scale study, 4.0-5.0% extruded flaxseed diet resulted in fewer days from first artificial insemination to conception in addition to fewer open days (Moallem, 2018). In addition, cows fed 2.7-3.2% of DM fishmeal from 24 days pre-partum to 109 postpartum had higher pregnancy rate (41.3 vs. 31.9%). Petit and Twagiramungu (2006) found that cows fed whole flaxseed to 120 d postpartum showed no embryo mortality. Feeding algae increased resumption of estrous at day 58 postpartum and pregnancy at first artificial insemination, and increased pregnancy per artificial insemination, which reduced days to pregnancy by 22 d compared with control cows (Sinedino *et al*., 2017). Collectively, the improvement in ovarian follicles and corpora lutea, embryo mortality, blood metabolites and hormones could explain higher reproductive performances in both extruded flaxseed and salmate groups.

The effects of supplementation extruded flaxseed (7.0%) and salmate (25 g/head/day) to lactating cows from three weeks pre-partum to 160 days postpartum on blood metabolites and hormone values are presented in Tables II. It was observed that the values of blood metabolites and hormones in extruded flaxseed, salmate and control group were within the normal range (Krogstad and Bradford, 2023). Of note, in the present trial, values of total protein (P = 0.088) and globulin (P = 0.053) were increased in extruded flaxseed group compared to salmate and control groups due to the anti-inflammatory properties of omega-3 fatty acids in flaxseed (Kra *et al*., 2021). Blood urea nitrogen (P = 0.0016) were increased in extruded flaxseed and salmate groups compared to control one. This is attributed to changes in feed digestibility and rumen fermentation (data not shown).

Extruded flaxseed diet resulted in lower (P < 0.0001) concentration of plasma NEFA and higher concentration of β-HBA compared to salmate and control groups. Conjugated linoleic acid has been shown to decrease NEFA mobilization from body fat stores leading to lower circulating NEFA values (Zhu *et al*., 2023). In addition, the alteration of rumen fermentation patterns might lead to increase βHBA production as an alternative energy source, especially during periods of NEB. The values of glucose and TG were decreased (P < 0.0001) in extruded flaxseed and salmate groups compared to control as a result of increasing milk production in those groups in addition to changes in milk composition and fatty acids profiles (Moallem *et al*., 2020; Beauregard *et al*., 2023). Besides, cholesterol, HDL and acetate values were higher in in extruded flaxseed and salmate groups compared to control as in other studies (Dail *et al*., 2011; Utama *et al*., 2018) due to the fat contents in flaxseed and salmate diets. In the regard of hormonal changes due to flaxseed and salmate diet; insulin, T4 and IGF-1 values were increased flaxseed group (P < 0.0001) whereas cortisol values were decreased. Such hormonal changes are consistent with other changes in plasma metabolites as lower glucose concentration and increased milk production in addition to alleviation of stress.

CONCLUSION

The results of extruded flaxseed (7.0%) or salmate (25 g/head/day) inclusion in diets of commercial lactating Holstein farm during transitional period till six months of lactation show notable positive effects on acceleration of ovarian activities in addition to significant increase of conception rate. Furthermore, the plasma metabolites and hormones values were within the normal ranges supporting body health and production.

ACKNOWELGMENT

The authors want to thank and acknowledge Deanship of Scientific Research, King Faisal University, Saudi Arabia for funding and support (GRANT5825).

Funding

The authors would like to acknowledge the support of the Research and Development Department of the Arabian Agricultural Services Company (Arasco).

IRB approval

The approval of the study was granted by the Ethical Research Committee of King Faisal University, Saudi Arabia.

Ethical approval

Animals care in the current trial was approved of the scientific research deanship ethical standards of King Fisal University (Ref. No. KFU-REC).

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Al-Masruri, H., Al-Zeidi, R., Al-Mufarji, A., Mohammed, A.A., Al-Madani, A. and Mohammed, H., 2022. Leverage of *Moringa oleifera* supplementation on performances, biochemical, and milk profiles in mammals. *Adv. Anim. Vet. Sci.,* **10**: 1887-2089. https://doi.org/10.17582/journal.aavs/2022/10.9.2043.2050

Al-Mufarji, A., Al-Suwaiegh, S. and Mohammed, A.A., 2023. Influence of organic *Moringa oleifera* leaves supplemented during gestation and lactation periods: Modulation of production efficiency, blood and metabolic parameters of ewes and lambs in subtropics. *Adv. Anim. Vet. Sci.*, **7**: 385-393. https://doi.org/10.17582/journal.aavs/2023/11.3.385.393

Al-Mufarji, A., Mohammed, A.A., Al-Zeidi, R., Al-Masruri, H. and Mohammed, A., 2022. Effects of *Moringa oleifera* on follicular development, blood and metabolic profiles of subtropical ewes during peripartum. *Adv. Anim. Vet. Sci.,* **10**:1706-1712. https://doi.org/10.17582/journal.aavs/2022/10.8.1706.1712

Ali, M.A., Alshaheen, T., Senosy, W., Kassab, A. and Mohammed, A.A., 2021. Effects of feeding green microalgae and *Nigella sativa* on productive performance and metabolic profile of Boer goats during peripartum period in subtropics. *Fresen.*

Environ. Bull., **30**: 8203-8212.

Al-Suwaiegh, S.B., Almotham, A.M., Alyousef, Y.M., Mansour, A.T. and Al-Sagheer, A.A., 2022. Influence of functional feed supplements on the milk production efficiency, feed utilization, blood metabolites, and health of Holstein cows during mid-lactation. *Sustainability,* **14**: 8444. https://doi.org/10.3390/su14148444

Ambrose, D.J., Kastelic, J.P., Corbett, R., Pitney, P.A., Petit, H.V., Small, J.A. and Zalkovic, P., 2006. Lower pregnancy losses in lactating dairy cows fed a diet enriched in a-linolenic acid. *J. Dairy Sci.,* **89**: 3066–3074. https://doi.org/10.3168/jds.S0022-0302(06)72581-4

Beauregard, A., Dallaire, M.P., Gervais, R. and Chouinard, P.Y., 2023. Lactational performance of cows fed extruded flaxseed in commercial dairy herds. *Anim.Open Space,* **2**: 100043. https://doi.org/10.1016/j.anopes.2023.100043

Cerri, R.L.A., Juchem, S.O., Chebel, R.C., Rutigliano, H.M., Bruno, R.G.S., Galvao, K.N., Thatcher, W.W. and Santos, J.E.P., 2009. Effect of fat source differing in fatty acid profile on metabolic parameters, fertilization, and embryo quality in high-producing dairy cows. *J. Dairy Sci.,* **92**: 1520–1531. https://doi.org/10.3168/jds.2008-1614

Dail, X.J., Wang, C., and Zhu, Q., 2011. Milk performance of dairy cows supplemented withrapeseed oil, peanut oil and sunflower seed oil. *Czech J. Anim. Sci.,* **56**: 181-191. https://doi.org/10.17221/1434-CJAS

Dere Yelken, H., Elci, M.P., Turker, P.F. and Demirkaya, S., 2024. Omega fatty acid ratios and neurodegeneration in a healthy environment. *Prostagland. Other Lipid Mediat*., **170**: 106799. https://doi.org/10.1016/j.prostaglandins.2023.106799

Di Meo, M.C., Salzano, A., Zotti, T., Palladino, A., Giaquinto, D., Maruccio, L., Romanucci, R., Rocco, M., Zarrelli, A., D'Occhio, M.J., Campanile, G. and Varricchio, E., 2023. Plasma fatty acid profile in Italian Holstein-Friesian dairy cows supplemented with natural polyphenols from the olive plant *Olea europaea* L. *Vet. Anim. Sci.*, **21**: 100298. https://doi.org/10.1016/j.vas.2023.100298

Djokovic, R., Kurćubić, V., Ilić, Z., Cincović, M., Fratrić, N., Stanimirović, Z., Petrović, M.D. and Petrović, M.P., 2013. Evaluation of the metabolic status of Simmental dairy cows in early and mid-lactation. *Anim. Sci. Pap. Rep*., **31**: 101-110.

Duncan, D.B., 1955. Multiple range and multiple Ftest. *Biometrics*, **11**: 1. https://doi.org/10.2307/3001478

Elbarbary, N.S., Ismail, E.A. and Mohamed, S.A., 2023. Omega-3 fatty acids supplementation improves early-stage diabetic nephropathy and subclinical atherosclerosis in pediatric patients with type 1 diabetes: A randomized controlled trial. *Clin. Nutr.,* **42**: 2372-2380. https://doi.org/10.1016/j.clnu.2023.10.007

Gonthier, C., Mustafa, A.F., Oullet, D.R., Chouinard, P.Y., Bertianne, R. and Petit, H.V., 2005. Feeding micronized and extruded flaxseed to dairy cows: Effects on blood parameters and milk fatty acid composition. *J. Dairy Sci.*, **88**: 748-756. https://doi.org/10.3168/jds.S0022-0302(05)72738-7

Gross, J.J. and Bruckmaier, R.M., 2019. Invited review: Metabolic challenges and adaptation during different functional stages of the mammary gland in dairy cows: Perspectives for sustainable milk production. *J. Dairy Sci.* **102**: 2828-2843. https://doi.org/10.3168/jds.2018-15713

Karageorgou, D., Rova, U., Christakopoulos, P., Katapodis, P., Matsakas, L. and Patel, A., 2023. Benefits of supplementation with microbial omega-3 fatty acids on human health and the current market scenario for fish-free omega-3 fatty acid. *Trends Fd. Sci. Technol*., **136**: 169-180. https://doi.org/10.1016/j.tifs.2023.04.018

Kesler, D.J., 2005. *Estrus synchronization systems: GnRH*. Proceedings, applied reproductive strategies in beef cattle November 12 and 13, 2005. Texas A and M University, College Station.

Kra, G., Nemes-Navon, N., Daddam, J.R., Livshits, L., Jacoby, S., Levin, Y., Zachut, M. and Moallem, U., 2021. Proteomic analysis of peripheral blood mononuclear cells and inflammatory status in postpartum dairy cows supplemented with different sources of omega-3 fatty acids. *J. Proteom.,* **246**: 104313. https://doi.org/10.1016/j.jprot.2021.104313

Krogstad, K.C. and Bradford, B.J., 2023. The effects of feeding α-amylase-enhanced corn silage with different dietary starch concentrations to lactating dairy cows on milk production, nutrient digestibility, and blood metabolites. *J. Dairy Sci.*, **106**: 4666-4681. https://doi.org/10.3168/jds.2022-23030

Leroy, J.L.M.R., Sturmey, R.G., Van Hoeck, V., De Bie, J., McKeegan, P.J. and Bols, P.E.J., 2013. Dietary lipid supplementation on cow reproductive performance and oocyte and embryo viability: A real benefit? *Anim. Reprod.,* **10**: 258-267.

Moallem, U., 2018. Roles of dietary n-3 fatty acids in performance, milk fat composition, and reproductive and immune systems in dairy cattle. *J. Dairy Sci.,*

101: 8641–8661. https://doi.org/10.3168/jds.2018-14772

Moallem, U., Lehrer, H., Livshits, L. and Zachut, M., 2020. The effects of omega-3 α-linolenic acid from flaxseed oil supplemented to high-yielding dairy cows on production, health, and fertility. *Livest. Sci.*, **242**: 104302. https://doi.org/10.1016/j.livsci.2020.104302

Mohammed, A.A. and Al-Suwaiegh, S., 2023. Impacts of *Nigella sativa* Inclusion during gestation and lactation on ovarian follicle development, milk composition as well as the blood and metabolic profiles of Ardi goat in subtropics. *Agriculture*, **13**: 674. https://doi.org/10.3390/agriculture13030674

NRC, 2001. *Nutrient requirements of dairy cattle*, 7th Rev. ed. Nat. Acad. Press, Washington DC.

Petit, H.V., 2002. Digestion, milk production, milk composition, and blood composition of dairy cows fed whole flaxseed. *J. Dairy Sci.,* **85**: 1482–1490. https://doi.org/10.3168/jds.S0022-0302(02)74217-3

Petit, H.V., Cavalieri, F.B., Santos, G.T.D., Morgan, J. and Sharpe, P., 2008. Quality of embryos produced from dairy cows fed whole flaxseed and the success of embryo transfer. *J. Dairy Sci.,* **91**: 1786-1790. https://doi.org/10.3168/jds.2007-0782

Petit, H.V. and Benchaar, C., 2007. Milk production, milk composition, blood composition, and conception rate of transition dairy cows fed different fat sources. *Can. J. Anim. Sci.,* **87**: 591–600. https://doi.org/10.4141/CJAS07027

Petit, H.V. and Twagiramungu, H., 2006. Conception rate and reproductive function of dairy cows fed different fat sources. *Theriogenology,* **66**: 1316–1324. https://doi.org/10.1016/j.theriogenology.2006.04.029

Pinedo, P. and Melendez, P.P., 2022. Liver disorders associated with metabolic imbalances in dairy cows, veterinary clinics of North America. *Fd. Anim. Pract.,* **38**: 433-446. https://doi.org/10.1016/j.cvfa.2022.07.004

Robinson, R.S., Pushpakumara, P.G., Cheng, Z., Peters, A.R., Abayasekara, D.R. and Wathes, D.C., 2002. Effects of dietary polyunsaturated fatty acids on ovarian and uterine function in lactating dairy cows. *Reproduction,* **124**: 119–131. https://doi.org/10.1530/rep.0.1240119

SAS, 2008. *SAS user's guide: Basics*. Statistical Analysis System Institute, Inc., Cary, NC,USA.

Santos, J.E.P., Bilby, T.R., Thatcher, W.W., Staples, C.R. and Silvestre, F.T., 2008. Long chain fatty acids of diet as factors influencing reproduction in cattle. *Reprod. Domest. Anim.,* **43**: 23–30. https://doi.org/10.1111/j.1439-0531.2008.01139.x

Senosy, W., Kassab, A.Y. and Mohammed, A.A., 2017. Effects of feeding green microalgae on ovarian activity, reproductive hormones and metabolic parameters of Boer goats in arid subtropics. *Theriogenology,* **96**: 16–22. https://doi.org/10.1016/j.theriogenology.2017.03.019

Senosy, W., Kassab, A.Y., Hamdon, H.A. and Mohammed, A.A., 2018. Influence of organic phosphorus on reproductive performance and metabolic profiles of anoestrous Farafra ewes in subtropics at the end of breeding season. *Reprod. Domest. Anim.,* pp. 1-10. https://doi.org/10.1111/rda.13183

Sinedino, L.D.P., Honda, P.M., Souza, L.R.L., Lock, A.L., Boland, M.P., Staples, C.R., Thatcher, W.W. and Santos, J.E.P., 2017. Effects of supplementation with docosahexaenoic acid on reproduction of dairy cows. *Reproduction,* **153**: 707–723. https://doi.org/10.1530/REP-16-0642

Underwood, J.P., Clark, J.H., Cardoso, F.C., Chandler, P.T. and Drackley, J.K., 2022. Production, metabolism, and follicular dynamics in multiparous dairy cows fed diets providing different amounts of metabolizable protein prepartum and postpartum. *J. Dairy Sci.,* **105**: 4032-4047. https://doi.org/10.3168/jds.2021-20996

Utama, D.T., Lee, S.G., Baek, K.H., Chung, W.S., Chung, I.A., Kim, D.I., Kim, G.Y. and Lee, S.K., 2018. Blood profile and meat quality of Holstein-Friesian steers finished on total mixed ration or flaxseed oil-supplemented pellet mixed with reed canary grass haylage. *Animal,* **12**: 426-433. https://doi.org/10.1017/S1751731117001707

Veshkini, A., Ceciliani, F., Bonnet, M. and Hammon, M.H., 2023. Review: Effect of essential fatty acids and conjugated linoleic acid on the adaptive physiology of dairy cows during the transition period. *Animal*, **17**: 100757. https://doi.org/10.1016/j.animal.2023.100757

Yang, Z.H., Gorusupudi, A., Lydic, T.A., Mondal, A.K., Sato, S., Yamazaki, I., Yamaguchi, H., Tang, J., Rojulpote, K.V., Lin, A.B., Decot, H., Koch, H., Brock, D.C., Arunkumar, R., Shi, Z.D., Yu, Z.X., Pryor, M., Kun, J.F., Swenson, R.E., Swaroop, A., Bernstein, P.S. and Remaley, A.T., 2023. Dietary fish oil enriched in very-long-chain polyunsaturated fatty acid reduces cardiometabolic risk factors and improves retinal function. *Science,* **26**: 108411. https://doi.org/10.1016/j.isci.2023.108411

Zachut, M., Arieli, A. and Moallem, U., 2011.

Incorporation of dietary n-3 fatty acids into ovarian compartments in dairy cows and the effects on hormonal and behavioral patterns around estrus. *Reproduction,* **141**: 833–840. https://doi.org/10.1530/REP-10-0518

Zachut, M., Dekel, I., Lehrer, H., Arieli, A., Arav, A., Livshitz, L., Yakoby, S. and Moallem, U., 2010. Effects of dietary fats differing in n-6:n-3 ratio fed to high-yielding dairy cows on fatty acid composition of ovarian compartments, follicular status, and oocyte quality. *J. Dairy Sci.,* **93**: 529–545. https://doi.org/10.3168/jds.2009-2167

Zhu, S.L., Gu, F.F., Tang, Y.F., Liu, X.H., Jia, M.H., Valencak, T.G., Liu, J.X. and Sun, H.Z., 2023. Dynamic fecal microenvironment properties enable predictions and understanding of peripartum blood oxidative status and non-esterified fatty acids in dairy cows. *J. Dairy Sci.,* **2023**: 302.